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*ACCELERATED COMMUNICATION*

# Pharmacological Characterization of Heterologously Expressed ATP-Gated Cation Channels ($P_{2X}$ Purinoceptors)

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**SUMMARY**

cDNAs encoding $P_{2X}$ purinoceptors from human bladder smooth muscle and from rat PC-12 cells were expressed in oocytes and human embryonic kidney 293 cells. Agonist potencies of 2-methylthio-ATP = 2-chloro-ATP = ATP ≥ 2′- and 3′-*O*-(4-benzoylbenzoyl)-ATP ≥ adenosine-5′-*O*-(3-thio)-triphosphate ≥ $P^1,P^5$-di(adenosine-5′) pentaphosphate ≫ ADP prevailed for both $P_{2X}$ purinoceptors. There were two main differences in agonist sensitivity between the two receptors. First, ATP was 10 times more potent at the receptor from bladder ($EC_{50}$, 0.8 μM) than at the receptor from PC-12 cells ($EC_{50}$, 8.2 μM). Second, α,β-methylene-ATP and L- and D-β,γ-methylene-ATP were agonists in cells expressing the bladder smooth muscle receptor ($EC_{50}$, 1–3 μM) but were ineffective in cells expressing the PC-12 receptor. The $P_2$ purinoceptor antagonists suramin, pyridoxal phosphate 6-azophenyl-2′,4′-disulfonic acid, and pyridoxal-5-phosphate acted similarly at both receptor forms, producing noncompetitive inhibition, with $IC_{50}$ values of 1–5 μM for suramin and pyridoxal phosphate 6-azophenyl-2′,4′-disulfonic acid and 10–20 μM for pyridoxal-5-phosphate. 4,4′-Dilsothiocyanatostilbene-2,2′-disulfonic acid distinguished receptor subtypes, producing potent inhibition of the bladder smooth muscle $P_{2X}$-mediated response, with an $IC_{50}$ value of 3 μM; it inhibited the PC-12 form by <40% at 100 or 300 μM. This study thus defines the pharmacological properties of homo-oligomeric forms of these two types of cloned $P_{2X}$ receptor channels.

$P_{2X}$ purinoceptors are cation-selective channels gated by extracellular ATP; they are present on many visceral and vascular smooth muscle types, as well as numerous neuronal and glial cell types (1, 2). Pharmacological characterization of $P_{2X}$ receptor subtypes based on agonist potency profiles in intact multicellular tissues has been problematic, for several reasons. First, there is variable metabolism of ATP and some of its analogues by ecto-ATPases. Second, the agonists often activate G protein-coupled metabotropic $P_{2Y}$-type purinoceptors in the same tissue. Third, there may be mixed populations of $P_{2X}$ subtypes (1, 3, 4). The first two problems can be obviated by making whole-cell recordings from dissociated cells, using rapid, "concentration-clamp" delivery of agonists and using the patch pipette to dialyze out cytoplasmic constituents necessary to drive G protein-coupled processes. Recently, these types of electrophysiological studies have been carried out on acutely dissociated smooth muscle cells (5, 6), as well as cultured autonomic and central neurons and glia (7–10). These studies revealed three distinct $P_{2X}$ purinoceptor phenotypes, i.e., an α,β-MeATP-sensitive, desensitizing, inward current characteristic of smooth muscle $P_{2X}$ receptors, an α,β-MeATP-insensitive, nondesensitizing, inward current characteristic of responses in the PC-12 pheochromocytoma cell line and superior cervical ganglion neurons, and an α,β-MeATP-sensitive, nondensensitizing, inward current that is observed in other neurons such as celiac and nodose ganglia.

The present study addresses the third problem mentioned above, namely the possible existence in a given cell of multiple molecular species of $P_{2X}$ receptors. Distinct cDNAs encoding ionotropic $P_{2X}$ purinoceptors have been isolated from rat vas deferens smooth muscle and from nerve growth factor-differentiated PC-12 cells (11, 12). More recently, a human homologue of the rat vas deferens $P_{2X}$ receptor has been cloned from human bladder smooth muscle (13). When it is expressed in oocytes or mammalian cells, activation of this purinoceptor results in robust currents due to the opening of cation-selective ion channels. This provides the opportunity to determine the pharmacological profile of individual molecular species of $P_{2X}$ receptors by heterologous expression. The present study was undertaken to define agonist and antagonist properties of homo-oligomeric forms of the smooth muscle and PC-12 $P_{2X}$ purinoceptors.

ABBREVIATIONS: α,β-MeATP, α,β-methylene-ATP; ATPγS, adenosine-5′-*O*-(3-thio)triphosphate; D-β,γ-MeATP, β,γ-methylene-D-ATP; BzATP, 2′- and 3′-*O*-(4-benzoylbenzoyl)-ATP; DIDS, 4,4′-dilsothiocyanatostilbene-2,2′-disulfonic acid; 2MeSATP, 2-methylthio-ATP; L-β,γ-MeATP, β,γ-methylene-L-ATP; AP5A, $P^1,P^5$-di(adenosine-5′) pentaphosphate; PPADS, pyridoxal phosphate 6-azophenyl-2′,4′-disulfonic acid; HEK, human embryonic kidney; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid.





## Materials and Methods

**Expression systems.** Human urinary bladder $P_{2X}$ cDNA (13), subcloned into the pBKCMV expression vector, and PC-12 $P_{2X}$ cDNA (12) (supplied by A. Brake and D. Julius, University of California, San Francisco), subcloned into the pBKCMV vector or the pcDNA1-Amp vector, were used for transfection of HEK 293 cells and for *in vitro* RNA transcription and injection into oocytes. For transient transfections, 1 ml of Optimem containing 1 μg of cDNA and 5 μg of Lipofectin were placed in a 35-mm Petri dish containing four coverslips on which HEK 293 cells were plated ($5 \times 10^5$ cells/coverslip); this medium was removed after 5–6 hr of incubation at 37° and replaced with normal culture medium, and recordings were made 12–48 hr later. Greater than 90% of cells from which recordings were made responded to ATP, but no currents in response to applied ATP (30 or 100 μM) were observed in nontransfected ($n > 60$) or mock-transfected ($n = 22$) HEK cells. Defollicuated *Xenopus* oocytes were injected with 50 ng of $P_{2X}$ cRNA and kept at 18° in physiological saline solution containing penicillin/streptomycin (11); recordings were made 2–6 days after injection. No currents in response to ATP or other agonists used in this study (0.3 or 1 mM) were recorded in noninjected oocytes.

**Electrophysiological recordings.** Two-electrode voltage-clamp recordings were made from oocytes using a Geneclamp amplifier (Axon Instruments); microelectrodes were filled with 3 M KCl (0.5–2 MΩ). External solution contained 96 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, and 0.1 mM $BaCl_2$; barium replacement of external calcium was used to prevent activation of endogenous calcium-activated chloride currents (11). Conventional whole-cell recordings were made from HEK 293 cells using an Axopatch 200 patch-clamp amplifier (Axon Instruments). Patch pipettes (4–7 MΩ) contained 145 mM potassium aspartate, 11 mM EGTA, 5 mM HEPES, and 5 mM NaCl; external solution contained 145 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, 2.5 mM $CaCl_2$, 10 mM HEPES, and 10 mM glucose. Agonists were applied using a fast-flow U-tube delivery system (14). Native and cloned smooth muscle $P_{2X}$ receptors from vas deferens smooth muscle exhibit strong desensitization (11, 15, 16) (see also Ref. 2) and very prolonged rundown of the response; therefore, we measured currents in response to activation of the bladder form of the $P_{2X}$ receptor, because rundown of the response is much less marked (Ref. 16 and this study). Reproducible responses were obtained by applying agonist for 2–10 sec at intervals of 10 min during oocyte recordings or for 2 sec every 4 min during HEK cell recording. Little or no desensitization of the PC-12 form of the $P_{2X}$ receptor occurs (2, 12, 17) (see Results); in these experiments, agonists were applied for similar durations but at intervals of 30–60 sec.

Agonist concentration-response curves were constructed by expressing currents as percentages of the maximal current evoked by ATP (typically 30 or 100 μM) in the same oocyte or HEK cell; all currents were recorded at a holding potential of −60 mV or −70 mV during recordings from oocytes and HEK cells, respectively. At low concentrations of agonist (<1 μM ATP), responses of the bladder purinoceptor expressed in oocytes showed slowly developing, steady state responses during the 2-sec agonist application; these appeared to be due to access problems associated with the large size of the oocyte and resulted in slight overestimation of the peak response for these low concentrations (e.g., see Fig. 1c). However, the overall shapes of the concentration-response curves and the calculated $EC_{50}$ values showed no significant differences between oocyte and HEK cell expression systems. Nevertheless, because of these resolution problems in the oocyte expression system, usually only two or three agonist concentrations were applied to any one oocyte, whereas complete curves (five or six concentrations) were obtained for each agonist in individual HEK 293 cells, for both forms of the receptor. This allowed us to obtain mean $EC_{50}$ values and to estimate 95% confidence intervals for agonists in HEK cells (see Table 1); $EC_{50}$ values for agonists in oocytes were obtained from the pooled data, as shown in Fig. 2a. Antagonists were applied in both the superfusate and the U-tube solution that contained the agonist; antagonists were superfused for 5–10 min before agonist application. Concentration-response curves for agonists and antagonists were fit by hyperbolic functions using GraphPad software (GraphPad, San Diego, CA). All data are means ± standard errors.

**Drugs.** Adenosine, AMP (sodium salt), ADP (sodium salt), ATP (magnesium salt), ATPγS (tetralithium salt), UTP (sodium salt), α,β-MeATP (lithium salt), D-β,γ-MeATP (sodium salt), BzATP (tetraethylamonium salt), and DIDS (disodium salt) were obtained from Sigma Chemical Co. 2MeSATP (tetrasodium salt), 2-chloro-ATP (tetrasodium salt), and L-β,γ-MeATP were from Research Biochemicals. Pyridoxal-5-phosphate monohydrate was obtained from Aldrich; AP5A (trilithium salt) was from Boehringer Mannheim, and PPADS and suramin were obtained from Bayer.

## Results

**Agonists.** Currents evoked in response to ATP and other purinoceptor agonists in oocytes or HEK 293 cells expressing the smooth muscle form of the $P_{2X}$ receptor desensitized in the continued presence of purinoceptor agonists, whereas the PC-12 form did not (Fig. 1; see also Refs. 11 and 12). The two expression systems yielded similar agonist concentration-response curves and $EC_{50}$ values (Fig. 2; Table 1). For both forms of the receptor, 2MeSATP, 2-chloro-ATP, and ADP were full agonists, whereas BzATP, AP5A, and ATPγS produced maximal responses that were about 65% of the maximal response to ATP (Fig. 2). Half-maximal concentrations ($EC_{50}$ values) for each of these agonists to activate the PC-12 form of the $P_{2X}$ receptor were approximately 10-fold greater than those for the human bladder form of the receptor (Table 1). The methylene-substituted ATP analogues α,β-MeATP, D-β,γ-MeATP, and L-β,γ-MeATP evoked little (<10% of maximal ATP current) or no current in oocytes or HEK 293 cells expressing the PC-12 form of the $P_{2X}$ receptor but were very effective agonists in oocytes and HEK cells expressing the human bladder form of the receptor (Fig. 2). For either form of the receptor, adenosine, AMP, and UTP (100 μM) evoked currents that were 0–6% of the maximal ATP current ($n = 3$–5 for each agonist).

**Antagonists.** Low concentrations of suramin (1 or 3 μM) produced approximately parallel, rightward shifts in the ATP concentration-response curve, but the shifts in the presence of higher concentrations were no longer parallel (Fig. 3). The antagonism by even high concentrations of suramin (30 or 100 μM) was readily reversed within 1 min of washout from HEK cells expressing the PC-12 form of the $P_{2X}$ receptor; in the case of the bladder smooth muscle receptor the effect had been washed out by the time the agonist could be reapplied (i.e., 10 min).

The $P_2$ purinoceptor antagonists PPADS, pyridoxal-5-phosphate, oxidized ATP, and DIDS (18–22) also inhibited ATP-evoked currents in oocytes or HEK cells expressing ei-

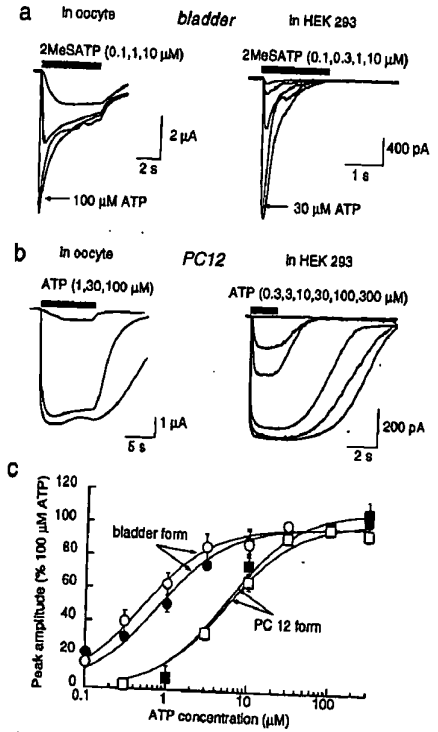


Fig. 1. Inward currents in response to activation of heterologously expressed $P_{2X}$ purinoceptors. a, Responses to activation of the $P_{2X}$ receptor cloned from human bladder smooth muscle, expressed in oocytes (*left*) or HEK 293 cells (*right*). Superimposed currents recorded from a single oocyte or HEK cell in response to increasing concentrations of 2MeSATP, as indicated, are shown. *Arrows*, current in response to a maximal concentration of ATP. b, Responses to activation of the $P_{2X}$ receptor cloned from the rat PC-12 cell line. Superimposed currents in response to increasing concentrations of ATP are shown. Note the pronounced desensitization of the smooth muscle form of the $P_{2X}$ receptor and the absence of desensitization with the PC-12 form of the receptor. c, Concentration-response curves for ATP obtained with the bladder (*circles*) and PC-12 (*squares*) forms of the receptor expressed in HEK 293 cells (*filled symbols*) and in oocytes (*open symbols*). Each value is the mean ± standard error of four to nine experiments.

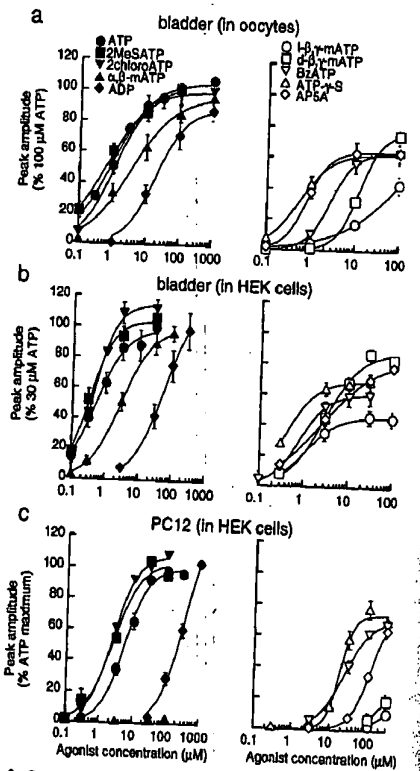


Fig. 2. Concentration-response relations for purinoceptor agonists at the bladder smooth muscle (a and b) and PC-12 (c) forms of the $P_{2X}$ purinoceptor. Each value is the mean ± standard error from four to nine separate experiments of the type illustrated in Fig. 1. *Left*, *lines* for full agonists [ATP, 2MeSATP, 2-chloro-ATP, and ADP for all and $\alpha,\beta$-MeATP ($\alpha,\beta$-*mATP*) for smooth muscle] are best fits to hyperbolic functions; Hill slopes for these lines ranged from 0.91 to 1.3. *Right*, *curves* for ineffective agonists are also best-fit hyperbolic functions. Note the ineffectiveness of methylene analogues at the PC-12 form of the receptor. $EC_{50}$ values for the bladder form expressed in oocytes (a), obtained directly from these curves, are as follows: ATP, 0.8 μM; 2MeSATP, 0.6 μM; 2-chloro-ATP, 0.8 μM; ADP, 34 μM; AP5A, 2 μM; BzATP, 0.6 μM; ATPγS, 3 μM; $\alpha,\beta$-MeATP, 3.6 μM; D-$\beta,\gamma$-MeATP, 1.4 μM; L-$\beta,\gamma$-MeATP, 2 μM. See Table 1 for additional details of values obtained in individual cells using the HEK expression system.

ther form of the $P_{2X}$ receptor; the inhibition by these antagonists was clearly noncompetitive and required more than 15–30 min for effects to reverse (data not shown). Therefore, we measured the inhibition of the current in response to a fixed concentration of ATP ($EC_{80}$ concentration) with increasing concentrations of antagonist, to obtain antagonist $IC_{50}$ values (Fig. 4). There were no clear differences in the actions of suramin, PPADS, pyridoxal-5-phosphate, or oxidized ATP to inhibit currents evoked by activation of either of the $P_{2X}$ receptor forms; $IC_{50}$ values for suramin and PPADS were approximately 1–5 μM for both receptor types and 10–20 μM for pyridoxal-5-phosphate (Fig. 4). Oxidized ATP produced only partial inhibition of $P_{2X}$-mediated currents (60% inhibition at the highest concentration examined) (Fig. 4). The inhibition by oxidized ATP was reversible within 15–25 min after washout, which is in contrast to its reported action as an irreversible receptor antagonist at the "pore-forming" $P_{2Z}$ purinoceptor (22). However, this distinction may simply reflect the different antagonist incubation procedures, in that Wiley *et al.* (22) observed covalent linking of oxidized ATP to $P_{2Z}$ purinoceptors in lymphocytes after 24 hr of antagonist application.

Curare has been reported to be somewhat effective in blocking the $P_{2X}$ current both in native PC-12 cells and in oocytes expressing the PC-12 form of the receptor (12, 23). However, we found that only very high concentrations of curare (1 mM) produced significant inhibition of the ATP-mediated current (Fig. 4); therefore, it is unlikely that this



TABLE 1

**Agonist $EC_{50}$ values**

Half-maximal concentrations ($EC_{50}$ values) were calculated as the concentration giving 50% of the maximal response for each concentration-response curve, generated in individual HEK 293 cells. Values are means ± standard errors for the numbers of individual experiments shown in parentheses.

| | $EC_{50}$ | |
|---|---|---|
| | Human urinary bladder | Rat PC-12 |
| | μM | |
| ATP | 0.9 ± 0.2 (6) | 7.7 ± 1 (9) |
| 2MeSATP | 1 ± 0.3 (7) | 3 ± 0.8 (8) |
| 2-Chloro-ATP | 0.5 ± 0.2 (5) | 2.5 ± 1.1 (5) |
| ADP | 73 ± 16 (5) | 222 ± 41 (5) |
| AP5A | 2.5 ± 1.2 (4) | 89 ± 7 (5) |
| BzATP | 0.7 ± 0.2 (5) | 23 ± 3 (6) |
| ATPγS | 3.1 ± 0.3 (4) | 21 ± 3 (6) |
| α,β-MeATP | 2.2 ± 0.3 (9) | ⩾100 (9) |
| D-β,γ-MeATP | 2.8 ± 0.6 (4) | ⩾100 (7) |
| L-β,γ-MeATP | 1.9 ± 0.6 (5) | ⩾100 (6) |

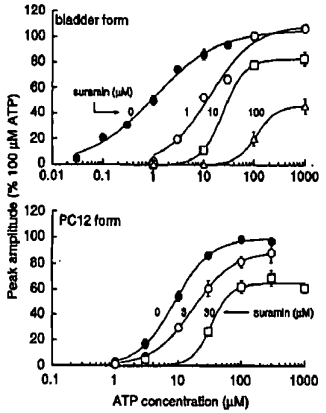


Fig. 3. Actions of suramin at the smooth muscle and PC-12 forms of the $P_{2X}$ purinoceptor. Each panel shows concentration-response curves for ATP in the presence of increasing concentations of suramin, as indicated. *Upper*, data for the bladder form of the $P_{2X}$ receptor, obtained in the oocyte expression system (four determinations for each value); *lower*, data for the PC-12 form of the receptor, obtained in the HEK cell expression system (eight determinations for values obtained in the absence of suramin and four determinations for values obtained in the presence of suramin).

small inhibition (<25%) represents a selective antagonism of the $P_{2X}$ purinoceptor.

DIDS, which is generally considered an inhibitor of anion transport, has also been shown to be an effective inhibitor of the native $P_{2X}$ purinoceptor in rat vas deferens (21); it showed a clear distinction between the smooth muscle and PC-12 forms of the receptor. Preliminary experiments with DIDS showed that a gradual, concentration-independent reduction in the ATP current occurred when this compound was superfused for >10 min; therefore, all measurements were made after 6 min in the presence of DIDS for both forms of the receptor expressed in HEK 293 cells. DIDS (100 μM) produced an 80% inhibition of the ATP-evoked current in HEK 293 cells, with an $IC_{50}$ value of about 3 μM. However, maximal concentrations of DIDS (100 and 300 μM) produced

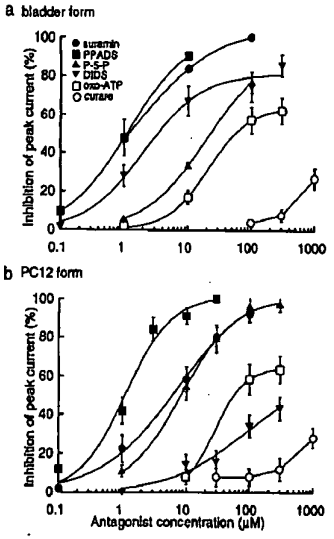


Fig. 4. Concentration-response curves for inhibition of current, produced by a fixed concentration of ATP, by $P_2$ purinoceptor antagonists at cloned $P_{2X}$ purinoceptors. a, Inhibition of current induced by 10 μM ATP at the bladder smooth muscle form of the $P_{2X}$ receptor. Data for suramin, PPADS, and pyridoxal-5-phosphate were obtained in the oocyte expression system, whereas data for DIDS, oxo-ATP, and curare were obtained in HEK 293 cells (four to six determinations for all values). b, Antagonism by the same antagonists of current induced by 30 μM ATP with the PC-12 form. All data were obtained from HEK cells transiently transfected with the PC-12 $P_{2X}$ receptor (four to eight determinations for all values).

<40% inhibition of the current evoked by ATP in cells expressing the PC-12 form of the receptor (Fig. 4).

## Discussion

The original classification of the $P_{2X}$ purinoceptor subtype was based on the relative potency of ATP and several structural analogues in assays of contractile or depolarizing actions on isolated whole tissues. This led to an agonist potency order of $\alpha,\beta$-MeATP ≫ 2MeSATP ≥ ATP becoming accepted as the general pharmacological definition of this receptor type (24). Although numerous subsequent studies on intact multicellular tissues yielded similar results, it has now become clear that such results were predominantly due to breakdown of ATP, 2MeSATP, and other hydrolyzable analogues by ectonucleotidases. When this activity is prevented the actions of $\alpha,\beta$-MeATP remain unaltered, whereas ATP and 2MeSATP become effective at 10–100-fold lower concentrations, thus changing agonist potency to 2MeSATP ≥ ATP > $\alpha,\beta$-MeATP. The $EC_{50}$ values for these agonists are then all in the low micromolar (1–10 μM) range (25) (also see Ref. 4). Whole-cell recordings from dissociated smooth muscle cells, nodose neurons, and celiac neurons give similar findings (5, 6, 10), supporting the conclusion that these agonist affinities and the rank order of potencies are characteristic of endogenous smooth muscle $P_{2X}$ purinoceptors as well as some neuronal $P_{2X}$ receptors. The present results on the

heterologously expressed $P_{2X}$ purinoceptor from human bladder smooth muscle directly confirm this pharmacological profile for smooth muscle $P_{2X}$ purinoceptors.

The present study allows direct comparisons between homo-oligomeric forms of the smooth muscle $P_{2X}$ receptor and the PC-12 form of this receptor, as well as between the *Xenopus* oocyte and mammalian HEK 293 cell expression systems, which are currently the two most commonly used expression systems for studying ligand-gated ion channels. We have found no obvious differences in responses of $P_{2X}$ purinoceptors expressed in either oocytes or HEK 293 cells (e.g., Figs. 1 and 2 and Table 1); such similarities suggest that additional proteins contributed by one or the other cell type used may not be critical for agonist/antagonist recognition. We might therefore ask the following: what are the distinguishing features of the two receptor types with respect to agonist and antagonist binding, and how do these properties in heterologous cells compare with their properties in native cells?

The clearest difference between the PC-12 and smooth muscle forms of the $P_{2X}$ receptor is the absence of agonist action of the methylene analogues $\alpha,\beta$-MeATP, D-$\beta,\gamma$-MeATP, and L-$\beta,\gamma$-MeATP at the PC-12 subtype, in contrast to their potent activation of the smooth muscle subtype. The differential sensitivity to $\alpha,\beta$-MeATP of native smooth muscle and PC-12 $P_{2X}$ purinoceptors is well documented (see the introduction) and was noted in the original reports on the cloning and expression of the rat vas deferens form and the PC-12 form of these purinoceptors (11, 12). This difference allows distinction between the smooth muscle form of the $P_{2X}$ receptor and one kind of neuronal type of this purinoceptor but does not allow distinction between the smooth muscle receptor and the $\alpha,\beta$-MeATP-sensitive neuronal $P_{2X}$ receptor (2, 4, 10). L-$\beta,\gamma$-MeATP may prove to be more useful for differentiation of smooth muscle $P_{2X}$ receptors from all forms of neuronal $P_{2X}$ receptors, because this agonist has been found to be ineffective in those neurons that do respond to $\alpha,\beta$-MeATP (e.g., celiac and nodose ganglia), as well as those that do not (e.g., PC-12 cells and rat superior cervical ganglion neurons) (26). All other agonists examined in the present study showed approximately similar rank orders of potency for the $P_{2X}$ receptor subtypes, although the $EC_{50}$ value for any one agonist to activate the PC-12 form of the receptor was approximately 10-fold greater than that for the bladder smooth muscle form (Table 1).

Of the $P_2$ purinoceptor antagonists used in this study, only DIDS was able to differentiate between the smooth muscle and PC-12 forms of the $P_{2X}$ receptor (Fig. 4). This finding may be of practical use for further delineation of $P_{2X}$ receptor subtypes in single isolated cells, and it will be interesting to determine whether the differential sensitivity observed in the present study will be maintained at native receptors in dissociated smooth muscle and autonomic and central neurons. However, the noncompetitive and virtually irreversible nature of this inhibition at the smooth muscle $P_{2X}$ receptor, the documented inhibition by DIDS of both $P_{2Z}$-like and $P_{2Y}$-like purinoceptors (21), and the more commonly known role of DIDS as an anion transport inhibitor are likely to limit the usefulness of DIDS as a more general $P_{2X}$ receptor antagonist.

The $IC_{50}$ values for suramin and PPADS to inhibit currents in response to activation of either $P_{2X}$ receptor ranged from 1 to 6 $\mu$M, whereas half-maximal inhibition by pyridoxal-5-phosphate occurred between 10 and 20 $\mu$M. None of these antagonists appeared to act in a competitive manner, with the exception of quite low concentrations of suramin (Fig. 3); only the effects of suramin were readily reversible. Even over this limited concentration range, it seems inappropriate to estimate and compare dissociation equilibrium constants for suramin, because in one case (bladder smooth muscle) the agonist action was strongly desensitizing and not at equilibrium. Oxidized ATP was a much weaker $P_{2X}$ purinoceptor antagonist, producing <60% inhibition at the highest concentration examined (300 $\mu$M). These results are in general agreement with a large body of data obtained from studies on native $P_{2X}$ purinoceptors in a variety of smooth muscle and neuronal tissues and serve to further highlight the need for development of more selective and, particularly, competitive $P_{2X}$ purinoceptor antagonists that will allow pharmacological differentiation among $P_{2X}$ receptor subtypes (1, 2, 4).

We next ask how closely the properties of the expressed receptors resemble those found in the tissues from which the receptors were cloned, because discrepancies might indicate the presence of ancillary proteins that contribute to the native receptors. Purinoceptor responses in rat, guinea pig, and human bladder smooth muscle have been characterized (16, 27–29); PC-12 cells have been studied by Nakazawa *et al.* (17, 23, 30). The properties of the human bladder form of the receptor expressed in oocytes or HEK 293 cells very closely resemble those of the native receptors; this is true with respect to the absolute concentrations of agonists that are effective, the rank order of agonists, and the effective concentrations of antagonists. The concordance is rather less in the case of the PC-12 form of the receptor, where cells (either oocytes or HEK 293 cells) expressing the cloned receptor are about 10 times more sensitive to all of the agonists than are native PC-12 cells. Such a difference could arise from different post-translational processing in the different cells or from the presence of additional proteins in the native cells.

The smooth muscle $P_{2X}$ receptor from rat vas deferens and its human homologue from bladder smooth muscle have approximately 50% sequence similarity to the PC-12 form of the receptor (11–13) (also see Ref. 2). These receptors show no sequence similarity to other proteins, and it has been suggested that they have two membrane-spanning domains, with most of the protein forming a large extracellular loop. This molecular architecture contrasts with that of other, functionally similar, ligand-gated channels activated by acetylcholine, 5-hydroxytryptamine, $\gamma$-aminobutyric acid, glycine, and glutamate, which have extracellular amino termini and at least three membrane-spanning domains (31). The pharmacological properties of those channels reflect their subunit composition; nicotinic channels are pentamers with up to four different subunits (31). The cloning of two cDNAs encoding the smooth muscle and PC-12 forms of the receptor makes it highly likely that related family members exist and, if the channels form multimers, pharmacological diversity may also be generated by heteropolymerization. Indeed, despite the similarities found here between the pharmacological properties of the cloned and native receptors, it remains to be shown that native channels either in smooth muscle or in PC-12 cells form homomultimers.



**Acknowledgments**

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